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STUDIES ON IMMUNITY IN ROCKY MOUNTAIN SPOTTED FEVER.*

FIRST COMMUNICATION.†

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IN these studies it has been our aim to ascertain with certainty some of the fundamental facts concerning immunity to this disease and to pass as rapidly as possible to the practical aspects of the question, namely, the study of serum prevention, serum therapy, and specific vaccination. Concerning these three important phases of the work, we report preliminary experiments which indicate the scope of our studies, the results of which will be reported more exhaustively in the future.

DIAGNOSIS OF THE EXPERIMENTAL DISEASE.

Before considering the experiments it seems desirable to point out the essential clinical and anatomical features of experimental spotted fever in order to show that the criteria for the recognition and differentiation of the disease are of a definite and convincing character. While diagnosis is usually easy concerning infections caused by organisms that may be cultivated and that show definite biologic characteristics, or that have a definite morphology, as in the case of certain protozoa, it would appear to be more difficult and liable to error when the organism is unrecognized and uncultivated.

The incubation period is definite and is never absent in animals that were fever free and that suffered no accident at the time of inoculation such as might be caused by puncture of the intestines or some accidental infection. Following intraperitoneal inoculation in the guinea-pig, monkey, or rabbit,[1] two to four or five days elapse before a distinct rise in the temperature occurs. The incubation period is from two to four days longer when the inoculation is subcutaneous. Its length also has a certain relation to the quantity of virus inoculated; when the minimum pathogenic dose is used it is often from one to three days longer than when several multiples of this dose are injected.

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[1] The rabbit has recently been found to be susceptible to the strains now cultivated in laboratory animals.

At the close of the incubation period the temperature rises to a maximum rapidly. Not infrequently it is normal (about 102.5) on one day, and on the next day, 105 or even 106.[1] More often, however, the first day of the febrile period is marked by a temperature of 103 to 104 with a more pronounced rise on the second and following days. The course is that of a continuous fever throughout, as in man. For working purposes it is satisfactory to take the temperature but once a day and it is immaterial whether this is done in the morning or afternoon. Sometimes the maximum temperature is not reached until the third or fourth day of fever. Usually the high point is about 106, although a temperature of 107.2 has been observed.

In fatal cases the duration of the fever is subject to variations, some dying after four or five days and others after eight or ten days of fever. Similarly animals which recover may exhibit fever for from six to ten days. In recovery subsidence of the temperature is usually gradual. Occasionally, however, a drop to normal is noted within 24 hours, and it may be slightly below normal for one or two days. In fatal cases the reduction is sudden, and a drop to 97 or 99 is an unfailing sign of impending death.

The most characteristic external signs of the disease are emaciation, a roseolar eruption, hemorrhages into and gangrene of the external genitalia. The roseolar eruption begins on the third to the fifth day of fever and is best seen on the external genitalia of guinea-pigs with white skins. If such animals are shaved it may also be detected on other parts of the body, especially the thighs, back and face. It is not always recognizable and escapes observation entirely on animals having dark skins. In males the scrotum begins to swell after from two to four days of fever and it is characteristic for the skin at this point to become densely infiltrated with blood; in the event that the animal lives long enough, i. e., in the event of recovery, the hemorrhagic areas become gangrenous and separate, leaving greater or less deformity of the scrotum. These phenomena are not absolutely constant but occur in a high percentage of the males. Apparently the swelling of the scrotum is due chiefly to congestion and edema of the underlying connective tissue, the tunica vaginalis and the epididymis. In females, the vulva becomes swollen and may show the roseolar eruption as stated; hemorrhage and gangrene of the vulva, however, are rather uncommon. Frequently the ears suffer from gangrene to a greater or less extent and it is the impression that this phenomenon now occurs more frequently than when the virus was first cultivated from man. Occasionally both ears fall away to their bases. Less frequently the soles of the feet undergo gangrene. In fatal cases the guinea-pig loses approximately one-third of its weight during the disease.

The most characteristic findings at autopsy, aside from the changes in the genitalia, are a greatly enlarged spleen and enlarged and hemorrhagic lymph glands with extreme congestion of the adjacent areolar tissue. Enlargement of the suprarenal glands is of frequent occurrence and they sometimes contain small hemorrhages.

[1] It has been the rule in taking the temperature of guinea-pigs to insert the thermometer as far as possible into the colon. If this distance is not more than two inches, it is usually because the colon is occluded by feces, and this condition is recorded at such times. In animals of 300 to 400 grams weight it is usually possible to reach a depth of about three inches, and in full grown guinea-pigs the thermometer can be inserted for almost its entire length. (This is the ordinary clinical thermometer.) A large number of observations indicate that the normal temperature of the guinea-pig, as taken in this way, lies between 102.2 and 102.6. A temperature above 103 would seem to be abnormal, although occasional groups of animals are encountered in which the temperatures may vary between 103 and 104.5, without any evident cause. This may persist for several days and, naturally, causes confusion in the recognition of spotted fever.

A valuable point for the corroboration of the diagnosis is the failure to cultivate any microorganism from the blood or organs of animals that have been killed during the course of the fever, or which have died as a natural result of the infection. This statement refers to typical cases, in which the clinical course indicates that there was no mixed infection, and in the case of autopsies it applies to those that are performed immediately or soon after death.

This negative bacteriologic finding has been constant through two years of work and although it is of the greatest importance as a diagnostic point, the clinical course of the disease is so characteristic that cultures are now resorted to only occasionally to prove the purity of the virus used for passage, and in experiments to serve as a check for animals in which there has been some departure from the normal course of the disease or in which the autopsy reveals unusual conditions. If the animals used for an experiment are free from fever when the experiment is begun and if following injection they or their controls exhibit the usual incubation period followed by a typical course of fever and the development of the cutaneous phenomena and if the findings at autopsy are as described above the experiments are sufficiently controlled for working purposes. This method has been found perfectly satisfactory and in two instances was sufficient for the recognition of adventitious epidemics, the presence of which was subsequently verified by bacteriologic methods.

In view of the fact, as stated later, that an attack of spotted fever, however mild, confers strong immunity, an "immunity test" is of value in determining whether animals which recover have or have not suffered from spotted fever. Our experience indicates that any used animal which develops spotted fever after the injection of 20 to 50 multiples of the minimum pathogenic dose of virus could not have had the disease in the first instance.

THE VIRUS.

For the major part of the work two strains of the virus have been used, one obtained in the spring of 1906 and the other in 1907. The first is known as the Bradley strain, the second as the Eddy strain. During the summer of 1906 the Bradley strain was grown alternately in monkeys and guinea-pigs and subsequently in guinea-pigs alone. The Eddy strain has been cultivated in guinea-pigs exclusively. The method of cultivation is that of the ordinary passage, the blood of an infected animal being injected intraperitoneally or subcutaneously into a healthy one. The Bradley strain has undergone approximately 125 passages. The two strains show about the same degree of virulence judging from the quantity of virulent blood required to produce infection. On this account they have been used more or less interchangeably, a record being kept of the strain used for each experiment.

It is important to know whether the virus used in these experiments is the same specifically, as when first obtained. We have the following evidence that it is: Animals that had recovered from the infection with the virus after it had been cultivated in the guinea-pig for one year were immune to the inoculation of fresh virus obtained from man in the spring of 1907. Of two strains, one obtained in 1906 and one in 1907, each confers immunity against the other. This, of course, is independent of the possibility that the virus has undergone variations in the character of its pathogenicity as a result of prolonged cultivation in the guinea-pig.

The defibrinated blood of injected animals, the blood being drawn from the carotid artery or the heart, is the source of the virus, and when the term "virus" is

used, it is understood that it refers to such blood. The virus has been standardized as accurately as possible in terms of the minimum pathogenic dose. It is impossible to deal with the minimum lethal dose because of the fact that minimum infective doses are about as likely to prove fatal as several multiples of such doses. In most instances the minimum pathogenic dose lies between 0.01 c.c. and 0.03 c.c. The former frequently fails to produce infection whereas the latter is nearly always infective. For quantitative work it is important to draw the virus with respect to the duration of the infection, because of the possibility that antibodies which may appear later in the course may lessen infectivity. As illustrating the importance of this consideration, two strains were lost in the spring of 1906 by waiting until the animals had died before attempting to perpetuate the disease in succeeding generations.

We have gained the impression that virus which is taken rather late in the disease is more likely to produce infection, if it is diluted several fold with salt solution.

It has happened a number of times that small doses were infective whereas larger doses of the same virus were not. Such an experience occurred recently when infected blood from a horse, drawn rather late in the disease, was injected into two guinea-pigs in doses of 1 c.c. and 5 c.c.; the former amount caused spotted fever whereas the latter did not. This was not due to lack of susceptibility on the part of the latter animal, inasmuch as it contracted the disease later when inoculated with virus from the guinea-pig. We are inclined to the view that the failure of large doses to infect under these circumstances is due to the comparatively large quantity of antibodies in the larger quantities of blood. It is true that the proportionate quantity of antibodies to the number of microorganisms in 5 c.c. is the same as in 1 c.c. of blood, yet when the smaller quantity is used the antibodies are subject to greater dilution and it is in accordance with the known action of other antibodies to assume that their effect on the microorganisms would be somewhat attenuated in this instance by their dilution. Also there are good general grounds for believing that antibodies may be fixed or destroyed by the tissues to a certain extent, and if this is true the residuum of effective antibodies, in proportion to the number of microorganisms, would be lower when small quantities of virus are introduced than when larger quantities are given.

For most purposes the injections have been intraperitoneal, although the subcutaneous route has been employed for certain experiments.

CONCERNING IMMUNITY IN MAN.

In so far as we have been able to learn, there are no data regarding the immunity of man to Rocky Mountain spotted fever, although our knowledge of the matter is limited to the conditions in the Bitter Root Valley in Montana. There is no authoritative example of two attacks of the disease in the same person in this locality. It is true that one or more individuals state their belief that they have had more than one attack, but such statements lack the confirmation of the local physicians who are experienced in the recognition of the disease.

The results obtained in the monkey and the guinea-pig suggest that man probably acquires a strong immunity as a result of an attack.

This is a question, however, which can be approached from the experimental side by a study of the serum of those who have recovered.

ACTIVE IMMUNITY IN THE MONKEY.

The fact that one attack of spotted fever renders the monkey immune to a second inoculation, performed some months later, has already been reported by one of us.[1] An additional experiment illustrating the same point may be cited briefly.

On May 20, 1906, the monkey, a large *M. rhesus*, received intraperitoneally 10 c.c. of defibrinated blood from the patient, Porter. After an incubation period of about two days its temperature rose rapidly to 105.3 in the vicinity of which it remained for three days, and then gradually returned to normal during the course of five days more. The animal showed no eruption, although his eyes became much reddened. His blood was not inoculated into other animals in order to confirm the diagnosis of spotted fever and on this account there naturally existed a good deal of doubt as to whether the monkey had been infected with spotted fever by the inoculation.

On February 1, 1907, the monkey was given an immunity test, a control monkey (No. 24) being inoculated with the same quantity of virus. The inoculation caused no rise in the temperature of the animal, whereas the temperature of the control, after a brief incubation period, rapidly rose to 106.1, near which it remained until the animal was killed, on the sixth day after inoculation.[2] On the second day of high fever the control presented a roseolar eruption of the skin of the perineum, which was hemorrhagic at the time it was killed. The blood of this animal produced spotted fever in guinea-pigs.

The experiment reported previously and the one just cited indicate that an attack of spotted fever confers immunity in the monkey, which in the last instance was present in a marked degree for nine months. The quantity of virus injected was very large, consisting of defibrinated blood and a dense emulsion of the liver, spleen, and kidneys of an infected guinea-pig, a total quantity of 15 c.c.

[1] Ricketts, "Further Observations on Rocky Mountain Spotted Fever, etc.," *Jour. Amer. Med. Assoc.*, 1906, 47, pp. 1067–69.

[2] It was the custom at that time to kill the monkeys during the height of the fever in order to obtain quantities of virus for further experiments.

ACTIVE IMMUNITY IN THE GUINEA-PIG.

In a previous article it was also stated that the guinea-pig that has recovered from spotted fever is thereafter immune to the disease, although the details of experiments were not given at that time.[1] This result has proved to be a rule to which no exception has been found among a great many tests. An illustrative experiment will be cited in this place and other instances are found through the remaining part of this report.

Guinea-pig 710 was inoculated with spotted fever by the larvae of an infected tick. Twelve days after the first larvae were seen to be attached, the temperature of the guinea-pig rose suddenly to 105.2, and on successive days was read at 105.3, 104.8, 104.3, 103.8, 102.3, and 102.2. This was a short course of fever and some doubt existed concerning the correctness of the diagnosis until the result of a transfer inoculation was known. On the day when the temperature of Guinea-pig 710 registered 104.3, 3 c.c. of blood were drawn from the heart and injected intraperitoneally into Guinea-pig 749. The latter passed through a typical course of spotted fever, the temperature being as follows on succeeding days after inoculation: 103.4, 103.4,—, 105.6, 104.8, 106.7, 106.4, 106, 104.6, 105.4, 104.4, 104, 104.3, 103, 103.5; recovery. The scrotum of 749 became hemorrhagic and sloughed in a typical manner, and the animal, furthermore, resisted a subsequent immunity test. After these experiments had proved beyond doubt that Guinea-pig 710 had been infected by the larval ticks, the animal was given an immunity test by the intraperitoneal injection of 2 c.c. of virus from Guinea-pig 781, which had been inoculated with the Eddy strain of the virus. On successive days the temperature of Guinea-pig 710 was as follows: 103.4, 103, 102.6, 102.4, 102.3, 102.5, 102.7, 103.1, 102.3, ——, 103.6.

A good control for the experiment cited is found in the history of Guinea-pig 705 which also was used to determine whether or not the infected female tick transmits the disease to her progeny. Twenty-two days after the larvae of an infected tick were placed with the animal, the latter had four days of moderate fever: 104.3, 104.5, 103.1, 104.1, 102.1. This event suggested that the guinea-pig may have been given a mild infection by the larvae, but this was by no means certain

[1] Ricketts, *loc. cit.*

because the animals, confined and often exhausted as they are during such experiments, sometimes exhibit abnormal temperatures for one or several days which is entirely independent of infection with spotted fever. About three months later this animal received an injection of 2.5 c.c. of Bradley virus with the following result: 102, 102.5, 102.8, 105, 105.1, 104.7, 104.8, 103.9, 99.6; death. The figures represent the temperature of the animal on successive days. On the seventh day after inoculation the vulva became hemorrhagic; the autopsy showed typical changes in the spleen and lymph glands. Cultures were sterile.

It is a matter of first importance that a mild attack of spotted fever results in the formation of strong immunity. Usually, when small doses of virus are given, the animals either react with a typical and severe attack of spotted fever or they show no sign of infection whatever and in the latter instance it has been the custom to use them as duplicates in the course of routine passage. Occasionally, however, such animals have been found to be resistant to infection. For example, Guinea-pig 1064, which in a particular experiment had been injected with 0.01 c.c. of infected serum showed no disturbance other than one day of distinct fever, the following being the record of the temperature: 103.8, 102.8, 102.4, 102.8, 102.6, 104.8, 102.6, 103.2, 102.8, 103.2. Nine days after the first injection, it received intraperitoneally 1.25 c.c. of virus, diluted with salt solution, which had been kept in the ice-chest for five days. Although a control developed the disease, the animal was undisturbed by the inoculation, exhibiting normal temperature. A second immunity test was given 15 days later with the same result.

One might be inclined to explain such occurrences by the assumption that occasional guinea-pigs are immune to the disease naturally. Two conditions, especially, argue against the correctness of this assumption: First we have found no guinea-pigs which are immune to the infection when inoculated with a moderate quantity of the virus taken from an animal early in the disease, the minimum quantity necessary for infection being in the neighborhood of 0.02 or 0.03 c.c. Second, it is not difficult to repeat the result just quoted by injecting suitable quantities of virus and immune serum, the existence of active immunity being proved by an immunity test which is given after the

passive immunity, established by the injection of the immune serum, has disappeared. This will be referred to again under the subject of "protective inoculation."

The active immunity is probably of long duration. Animals have been found to resist infection with excessive doses of virus more than a year after they suffered from the disease. The occurrence of hereditary immunity, to be referred to below, also indicates the profound change which the disease produces in the guinea-pig as well as the permanent character of the immunity.

There can be little doubt that the cause of the active immunity lies in the anti-infectious properties which may be demonstrated in the blood and serum of animals which recover, and perhaps also in the acquired power of the tissues of the immune animal to produce additional antibodies readily when fresh virus is introduced at a subsequent time. The properties of the serum are considered below under "Passive Immunity." At present we have no means of deciding whether the antibodies are antitoxic, germicidal or opsonic. Theoretically all three may be represented.

HEREDITARY IMMUNITY.

The offspring of a female guinea-pig that has recovered from spotted fever are endowed with a strong and protracted immunity. This has been demonstrated many times and the resistance is transmitted regardless of the degree of infection of the female parent, a fact which is shown in the case of certain "vaccinated" female parents which exhibited a minimum febrile reaction. The influence of the immune male in the transmission of the immunity has not been investigated. Illustrative experiments will be described.

The female guinea-pig 663 was infected by male tick No. 5 on June 30, 1907. The following was the course of the temperature: 101.6, 102.4, 102.2, 103.7, 104, 105.4, 105.4, 105.2, 104.9, 104.6, 104.7, 103.8, 102.7; recovery. She gave birth to one young on October 14. On November 27, when the young animal was about six weeks old, it was injected intraperitoneally with 1 c.c. of third-day virus (Bradley strain). It had been with the parent continuously until injected. The following daily temperatures were recorded: 103, 103.6, 103.9, 103.2, 103, 102.5, 103.4, 103.1, 103, 103.8, 103,

103.8, 102.6, 103.1, 102.7.[1] A control guinea-pig of the same age, from a normal female, inoculated with the same dose of virus, showed the following daily temperatures: 103, 102.8, 102.8, 105.6, 107, 106.3, 106, 104.4, 104.5, 103.2, 100.2; death. The anatomical changes at autopsy were typical of spotted fever.

This experiment is important as showing that a female may transmit the immunity to her young although her infection antedated the period of her pregnancy by several months; that is, the cells of the embryo were not stimulated to form protective substances by the presence of virus; only the germ cell could have been subjected to such an influence.[2]

The following "exchange experiment" indicates that the inherited immunity does not depend entirely on the milk which the young derive from the immune parent.

Immune young which sucked immune parent.—Guinea-pig 938, 30 days old. Sucked for 14 days, was then removed, and 16 days later was inoculated. Had no distinct rise in temperature. Guinea-pig 939, 34 days old. Remained with parent until inoculated. No distinct fever.

Immune young which sucked normal parent.—Guinea-pig 935, 29 days old. Remained with normal parent until inoculated. No distinct fever followed. Guinea-pig 936, 34 days old. Was with normal parent for 26 days. No fever followed inoculation.

Normal young which sucked immune parent.—Guinea-pig 937, 30 days old. Course of temperature following inoculation: 102.8, 102.4, 104.2, 105.3, 105.5, 105.6; death. Autopsy: typical of spotted fever.

Normal young which sucked normal parent.—Guinea-pig 940, 29 days old. Daily temperatures following inoculation: 102.4, 102.5, 103.8, 104.5, 105, 105.1, 98.7; death. Autopsy: typical of spotted fever.

The duration of the inherited immunity has not been definitely determined. In one instance it was present two and one-half months and in another three months after birth.

[1] In our experience the young guinea-pig frequently has a higher average temperature than the adult and it is subject to greater fluctuations.

[2] Spotted fever in the guinea-pig is strictly an acute infection. The blood of the animal which recovers is never infective for other animals, but on the contrary is protective, as stated later.

We have not yet studied the character of the inherited immunity. In the event that the infection of the parent occurred before her impregnation, there is reason to think that the immunity of the young is passive in character. If infection occurs during pregnancy, there may be opportunity for the establishment of active immunity on the part of the embryo. However, infection occurring at this time usually results in abortion. In the event that the condition is one of passive immunity, it differs from the passive immunity established by the injection of immune serum by its greater duration. The immunity conferred by the injection of 1 c.c. of immune blood into a guinea-pig has disappeared largely after the lapse of 30 days. It is to be noted, however, that the offspring of an immune female may contain in their body fluids a much larger quantity of protective substances than is introduced in 1 c.c. of immune blood, and that the elimination of the larger quantity may require a much longer time. This subject will be investigated further.

PASSIVE IMMUNITY.

The whole blood, defibrinated blood, and serum of animals that have recovered from spotted fever possess strong protective powers when injected with virus into healthy guinea-pigs. For the sake of convenience immune defibrinated blood, rather than serum, has been used for most of the experiments, although it has been determined that the protective powers lie in the serum. When the term "blood" is used, it refers to defibrinated blood.

For some of the experiments on passive immunity the blood of animals which have recovered and which have been subjected to no further treatment has been used; in other cases so-called "hyper-immune" blood was employed. This is the blood of guinea-pigs that after recovery received a series of injections of infected blood with the hope of increasing the quantity of protective substances. We doubt, however, whether such a practice is of much service in producing an increase in the amount of antibodies. In two instances the blood of guinea-pigs which were treated in this way showed an actual decrease in their protective power which fell below that of other immune animals that had not received fresh injections of virus. Both of these animals were bled repeatedly during the immunizing

process, however, and the decrease may have been due to the dilution accompanying the restitution of the blood. As a rule, the blood of guinea-pigs that have recovered recently has shown as great protective powers as that of "hyper-immune" animals. It is not unlikely that the concentration of antibodies in the blood of the immune animal decreases with time. There is a possible source of error in determining this point, however, in that a number of successive bleedings of the same guinea-pig brings about a dilution of the protective substances in his body simulating a decrease by elimination or destruction within the body. Experiments bearing on this point have not been brought to completion.

The blood of guinea-pigs that have recovered from the disease recently protects in doses of from 0.1 to 0.3 or 0.4 c.c. against infection with 1 c.c. of third-day virus, a quantity which represents at least from 30 to 60, and in some instances 100, minimum pathogenic doses. This result is obtained when the immune blood and virus are mixed before injection, the inoculations being intraperitoneal. Preliminary experiments indicate that the protection is not so pronounced when the two are injected into different parts of the body at the same time, although the difference is not great

The following experiment gives an approximate idea of the duration of the passive immunity when 1 c.c. of immune blood is injected subcutaneously (Table 1). The immune blood in this instance was a

TABLE 1.

DURATION OF PASSIVE IMMUNITY.

Guinea-pig	Interval between Injection of Serum and Virus	Quantity of Virus Injected	Result
1001	20 days	1.0 c.c.	No fever
1000	33	1.0	Slight fever for 5 days
1002	38	1.0	Moderate fever for 5 days
1003	45	1.0	Severe fever for 9 days
1004	49	0.5	Severe course of fever. Killed
1005	55	0.03	Became infected after an incubation period of one week

mixture, obtained from three immune guinea-pigs (862, 878, 778), two of which had recovered from spotted fever one month previously and the third about three months previously. One-tenth cubic centimeter protected againct [1 c.c. of third-day virus in controls, the mixtures being injected intraperitoneally. The experiment animals

received the virus subcutaneously at different periods following the injection of the immune blood.

The relation of the duration of the passive immunity to the quantity of immune serum injected has not been determined.

The converse of this experiment was performed also; a number of guinea-pigs were inoculated subcutaneously with 1 c.c. of virus each, and the subsequent period determined at which 1 c.c. of immune blood would prevent the development of spotted fever (Table 2). In this case the immune bloods were drawn immediately before injection and they came from different immune animals; hence they probably were not of uniform value, though certainly not differing greatly.

TABLE 2.

PROPHYLACTIC EFFECT OF IMMUNE BLOOD WHEN INJECTED SUBSEQUENT TO VIRUS.

Guinea-Pig	Interval between Injection of Virus and Immune Blood	Result
851.....	1 day	One day of distinct fever which may have been due to other causes
852.....	2 days	The same result, the single day of fever occurring on the same day
853.....	3	The same result
854.....	4	Three days of high fever, reaching 106.5, followed by three days of low fever (about 104)

The experiment shows protection against 30 to 60 pathogenic doses at the least, when 1 c.c. of immune blood is given three days after the injection of the virus. From this time the injections of immune blood could be considered to have only a curative effect, and this will be mentioned later.

The results of these experiments on protection by means of immune blood or serum, of which we have abundant confirmation, would seem to have an important practical bearing. There is good reason to believe, on the basis of the results obtained, that serum prophylaxis of man is feasible provided sufficient quantities of a serum of reasonable strength can be prepared. Manifestly it would operate successfully only in case the inhabitants of infected districts who are bitten by ticks would report for prophylactic injections within two or three days after receiving the bite. If the results obtained with the guinea-pig apply also to man, the serum when given in sufficient quantity would ward off the danger pertaining to the recent exposure, but probably could

not be considered protective for a period longer than three or four weeks. A subsequent exposure would require another injection of serum.

We have been able to infect the horse with spotted fever recently and are now engaged in a study of the protective power of the serum obtained after the recovery of the animal. Preliminary experiments indicate that its value differs little from that of the guinea-pig, hence there is reason to believe that a protective serum in desirable quantities will be available.

AN EXPERIMENT IN SERUM THERAPY.

We have made preliminary observations on the curative value of the immune blood from the guinea-pig and shall describe an experiment which seems to indicate that its therapeutic power is not of high degree (Table 3).

This is a continuation of the passive immunity experiment described on p. 232, in which equal quantities (1 c.c.) of virus were inoculated into a number of guinea-pigs and the effect of the immune blood noted when given at subsequent periods. Inasmuch as the remaining animals began to show fever on the fourth day after inoculation, it became thereafter an experiment to determine the curative value of the immune blood.

It had been determined in other experiments that the normal blood of the guinea-pig has no protective power against spotted fever. Thus Guinea-pig 884 received intraperitoneally a mixture of 1 c.c. of normal blood and 0.3 c.c. of third-day virus and died of spotted fever after nine days. Also Guinea-pig 886 received 1 c.c. of normal blood and 0.05 c.c. of virus and exhibited the course characteristic of spotted fever, including hemorrhage into and sloughing of the scrotum. It resisted an immunity test given two months after recovery.

Inasmuch as both controls in this experiment recovered, the influence of the immune blood can be determined only by its effect on the duration and severity of the fever, the normal duration of which in this instance was approximately six days. With this as a basis of comparison, it is seen that 2 c.c. of immune blood given on the first day of fever exercised but slight if any influence on the course. Five cubic centimeters given on the first day quite positively shortened the course

since there were only three days of severe fever. One cubic centimeter given daily had no pronounced influence, although the temperature for the last three days was lower than in the controls. Two cubic centimeters given daily beginning with the second day of fever may have been a factor in the lower temperature during the last three days.

TABLE 3.

THE CURATIVE POWER OF IMMUNE BLOOD.

Virus injected December 4, 1907, subcutaneously.
Injections of immune blood subcutaneous on dates indicated.
Guinea-pigs designated by numbers, 855, etc.

Dec.	Temp. 855	Immune blood	Temp. 856	Immune blood	Temp. 857	Immune blood	Temp. 858	Immune blood
5	103.8		103.4		104.		103.9	
6	103.4		104.		103.7		103.4	
7	103.5		104.2		104.9		104.8	
8	103.6		103.		103.7		103.1	
9	105.6	2 c.c.	105.8	5 c.c.	105.	1 c.c.	105.8	
10	105.5		105.		105.7	1	106.	2 c.c.
11	105.7		105.		106.3	1	105.7	2
12	104.5		103.4		104.8	1	104.6	2
13	104.2		103.9		104.2	1	104.2	2
14	.....		.....		104.	1	104.8	2
15	102.2		102.5		102.9		103.9	
16	102.3		102.3		103.1		103.6	
17	102.2		102.		102.5		103.5	
18	102.6		102.6		103.7		103.3	
19							103.	
20							102.3	
	Recovery		Recovery		Recovery		Recovery	

Dec.	Temp. 859	Immune blood	Temp. 860	Immune blood	Controls*	
5	103.3		103.4		103.9	103.6
6	104.		103.7		102.7	104.
7	104.4		104.2		104.	104.
8	103.7		103.6		103.5	103.7
9	105.6		104.3		105.2	106.4
10	106.4		106.		106.3	106.2
11	106.8	2. c.c.	106.2	3. c.c.	106.	105.7
12	106.	2	105.2	3	105.4	105.1
13	105.8	2	105.3	3	105.6	105.8
14	105.2	2	105.8	3	.....	.....
15	103.6		103.9		103.2	103.
16	103.2		104.		103.4	103.5
17	102.7		103.1		103.	103.
18	102.8		102.7		102.7	103.1
19			103.3			
20			103.			
	Recovery		Recovery		Recovery	Recovery

* Inoculated with 1 c.c. of virus alone.

The same amount given daily, beginning on the third day of fever, had no effect, and a similar result is noted when the amount was increased to 3 c.c. daily.

The experiment cited seems to show quite positively that the immune blood exercises little influence on the disease unless it can be

given early and in large quantities. An important practical difficulty arises here, for the disease in man is rarely recognized until the eruption appears, which may be at any time from the second to the fifth day of fever. Physicians who are experienced in the diagnosis of the infection often make a probable diagnosis of spotted fever on the first or second day of sickness, in the spring of the year, in the case of patients that reside in infected districts. The probable diagnosis should be facilitated if a history of tick bite within one to two weeks preceding the onset of symptoms is given. In such cases, a large quantity of the serum given immediately may exercise a modifying influence on the course of the infection; in the event that the probable diagnosis was incorrect, there would be no likelihood of harm as a consequence of the injection of the serum.

The curative value of the immune serum from the horse is being studied carefully; inasmuch, however, as the preliminary tests have shown that it possesses about the same power as the blood of immune guinea-pigs, it is not anticipated that the results will be very different.

It is not probable that a serum of greater power than that afforded by the animal which has recovered from spotted fever, will be obtained until the microorganism can be grown in desirable quantities in artificial cultures. During the course of the infection there is already in the animal's body such a quantity of the organism that 0.02 or 0.03 c.c. of his blood is infective, and at present there is no means of increasing this amount for subsequent injections.

PROTECTIVE INOCULATIONS.

For more than a year occasional attempts have been made to attenuate the virus of spotted fever by desiccation, by the addition of glycerin, and by means of heat, so that it would be suitable for vaccination. Desiccation for this purpose has proved to be utterly unreliable and has been abandoned. The experiments will not be described further than to say that the principle followed was to begin the injections with comparatively large quantities of virus that had been dried in a vacuum over sulphuric acid for a longer period than was required to kill all the organisms, and for succeeding injections to use smaller quantities of virus which had been dried for shorter periods, passing finally to minute amounts of fresh virus. Infection eventually

resulted in practically all the animals, and although it may in the end be possible to produce immunity in this way, the method is so tedious, long, and uncertain, that it appears to have only theoretical interest.

The value of glycerin and low degrees of heat as attenuating agents is still being studied.

No systematic attempt has been made to determine the vaccinating properties of minute doses of the virus. As a rule a minute quantity either produces frank infection or causes no disturbance whatever, and in the latter case the animals are, in nearly all instances, susceptible to reinoculation. Occasional exceptions have been encountered, however. Such an animal (Guinea-pig 1064) was referred to under the discussion of active immunity (p. 227). In this instance marked active immunity developed, although the animal had but one day of fever. On account of the uncertainty as to what the virus will do when injected in quantities which approximate the minimum pathogenic dose, it is manifest that minute doses cannot be utilized for practical vaccination.

On account of the protective power which immune blood possesses, the possibility of mixed immunization or "sero-vaccination" seemed promising and we have devoted some time to the study of this subject.

At the beginning of the work three plans of inoculation were adopted: First, the immunizing effect of a single mixture of immune blood and virus. Second, the effect of repeated injections given at short intervals, the ratio of the quantity of the immune blood to the virus being gradually decreased until pure virus was injected. Third, the same as the second plan, except that longer intervals were allowed to intervene between the injections. The ultimate object in all three methods was to establish active immunity without causing severe infection. This was found to be readily possible with regard to the second and third methods, although the development of immunity in most instances was characterized by a mild and brief febrile reaction some time during the course of the injections. These methods, however, are protracted and tedious and are more of theoretical than practical interest, particularly since a high degree of immunity can also be established by a single injection of virus and immune blood when the two are used in proper proportions.

We shall therefore limit our description to certain experiments in

which immunity was established in one instance by two (Table 4), and in another by one, injection.

TABLE 4.

THE IMMUNIZING EFFECT OF TWO INJECTIONS.

First injection, consisting of virus mixed with different quantities of immune blood. Injections intraperitoneal. Immune blood from two animals (765, 766) that had recovered spontaneously.

No. of Experiment Animal	Virus	Immune Blood	Result
789	1 c.c.	1.5 c.c.	No fever
790	1	1.0	" "
791	1	0.7	" "
792	1	0.3	" "

Second injection, consisting of 1 c.c. of virus, given 25 days after the first.

Date	Guinea-pig 789	Guinea-pig 790	Guinea-pig 791	Guinea-pig 792
Dec. 2	102.0	102.2	102.2	102.4
3	102.3	103.	103.	103.
4	102.4	102.6	102.7	102.8
5	102.1	102.6	102.3	103.
6	101.8	102.6	102.4	104.7
7	103.6	103.4	103.	105.5
8	102.8	103.8	103.4	104.
9	102.8	103.2	104.2	104.8
10	103.	103.4	103.9	103.3
11	103.8	103.7	104.	94.4
12	102.7	102.7	102.2	Death

Following the second injection all the animals showed a certain amount of febrile reaction, although in the first three the rise in temperature was no greater than often encountered from other causes, such as minor injuries to the colon by the thermometer.

It was attempted to learn the degree of immunity which had been established in 789, which showed a minimum reaction, and in 791 which showed a more pronounced though mild reaction, by testing the protective power of their defibrinated bloods, in two consecutive experiments (Tables 5 and 6).

The first test, shown in Table 5, is not very satisfactory, particularly regarding the animals injected with the blood of Guinea-pig 791. Guinea-pig 960 which received the largest dose of this blood (1 c.c.) ran a distinct course of fever, the cause of which may not be open to determination. When sufficient time has elapsed for the passive immunity, which was conferred by the immune blood, to be eliminated, the protective power of its blood will be ascertained, and if this proves to be of sufficient strength it will have to be concluded that the animal suffered from a moderately severe attack of spotted fever, in spite of

TABLE 5.

THE PROTECTIVE POWER OF THE BLOODS OF THE VACCINATED GUINEA-PIGS 789 AND 791.

Test 1, performed 25 days after the second injection.

Virus, 1 c.c.+varying quantities of immune blood.

	IMMUNE BLOOD FROM GUINEA-PIG 789			IMMUNE BLOOD FROM GUINEA-PIG 791			CONTROLS, RECEIVING 1 C.C. OF VIRUS EACH†	
Date	1 c.c. No. 957	0.7 c.c. No. 958	0.3 c.c. No. 959	1 c.c. No. 960	0.7 c.c. No. 961	0.3 c.c. No. 962	No. 965	No. 966
Dec. 27	102.8	102.8	103.0	103.0	102.8	102.7	102.4	103.6
28	102.9	103.1	103.2	103.5	103.3	102.8	102.6	103.8
29	102.6	103.	104.3	103.8	104.	102.8	102.4	102.8
30	102.6	103.6	105.2	104.	104.	103.	103.	103.2
31	102.4	103.5	103.9	103.5	103.4	103.1	104.4	104.
Jan. 1	.....	.....	.....	.....	.....	.....	105.2	105.4
2	102.4	103.	104.8	104.7	103.2	102.7	105.4	105.5
3	.....	.....	.....	.....	.....	.....	Killed	Killed
4	103.2	104.4	104.	104.3	103.2	102.8		
5	102.8	104.3	102.8	104.5	102.5	102.4		
6	.....	.....	.....	.....	.....	.....		
7	102.6	104.	103.6	104.	102.3	102.2		
8	102.4	103.4	104.3	104.7	102.4	102.4		
9	101.6	103.5	103.2	104.8	102.3	102.6		
10	103.2	103.1	104.3	103.9	103.	103.4		
11	.....	103.5	104.	102.8	.....	.....		
	Recovery	Recovery	Recovery*	Recovery	Recovery	Recovery		

* Guinea-pig 959 died two weeks later, showing pneumonia and cheesy abscesses.

† Guinea-pigs 965 and 966 were "passage" animals and they were killed on January 2 in order to perpetuate the strain in other guinea-pigs.

TABLE 6.

THE PROTECTIVE POWER OF THE BLOODS OF THE VACCINATED GUINEA-PIGS 789 AND 791.

Test 2, performed 29 days later than the first test, and 54 days after the second immunizing injection was given.

Virus, 1 c.c.+varying amounts of the immune bloods.

	IMMUNE BLOOD FROM GUINEA-PIG 789			IMMUNE BLOOD FROM GUINEA-PIG 791			CONTROLS= VIRUS ALONE	
DATE	0.6 c.c. No. 1074	0.4 c.c. No. 1073	0.2 c.c. No. 1072	0.5 c.c. No. 1077	0.3 c.c. No. 1076	0.1 c.c. No. 1075	0.01 c.c. No. 1078*	0.05 c.c. No. 1079
Jan. 25	103.	103.8	103.6	102.8	102.6	103.2	102.8	103.2
26	102.8	103.2	103.	103.	101.8	102.	102.4	102.4
27	103.	103.2	102.6	102.6	102.2	102.	102.2	102.2
28	102.8	103.2	102.6	102.6	102.8	103.4	102.4	104.
29	102.6	102.4	102.	102.8	102.6	102.4	100.8	104.6
30	103.4	103.2	105.	102.8	103.2	.....	102.	105.2
31	103.4	104.4	104.8	102.8	.....	103.2	102.4	105.4
Feb. 1	104.	104.2	105.6	103.6	102.8	105.	103.2	105.8
2	104.4	105.3	104.4	102.7	103.8	104.8	102.4	105.2
3	103.6	104.8	104.	102.8	104.	103.8	102.4	104.
4	105.2	105.	104.4	102.	104.6	104.	103.2	99.8
5	104.4	104.	104.6	103.	104.	104.	103.4	Death†
6	104.4	103.4	104.4	102.8	103.4	102.8	103.6	
7	102.4	103.6	103.8	102.8	102.8	102.	102.2	
8	102.2	102.8	102.8	102.2	103.	102.	102.4	
9	102.4	102.	102.8	102.3	102.6	102.3	102.4	
	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	Recovery	

Duration of fever, 8 days | Duration of fever, 9 days | Duration of fever, 9 days | Little, or no fever | Duration of fever, 5 days | Duration of fever, 6 or 7 days

* Guinea-pig 1078 became infected when an immunity test was given on February 28, hence in this experiment the minimum pathogenic dose was greater than 0.01 c.c. of virus, but equal to or less than 0.05 c.c. as shown by the fatal course in Guinea-pig 1079.

† The genitalia were hemorrhagic, and the spleen was greatly enlarged.

the large dose of protective blood which it received, and in spite of the fact that one-third of this dose protected Guinea-pig 962.

Having in mind the animals which received 0.7 and 0.3 c.c. of the two immune bloods, the impression is given that the blood of 791 was somewhat more protective than that of 789.

The difference in the protective power of the two bloods is manifest in the second test. Thus, 0.5 c.c. of the blood of 791 prevented fever entirely, whereas 0.6 c.c. of 789 did not prevent the development of a distinct and rather high course of fever; 0.3 c.c. of the former permitted moderate fever for five days, while 0.4 c.c. of the latter allowed a marked febrile course of nine days; and the conditions are similar in regard to the third animal of each group (1075 and 1072). One probably could not come nearer to a comparison than to say that 0.6 c.c. of the blood of Guinea-pig 789 was equal to 0.1 c.c. of that from 791, since the severity and duration of the fever of the corresponding animals were about the same.

Comparison of the first test with the second indicates that the protective substances underwent a diminution in quantity in the interval of 29 days. In the first test the blood of Guinea-pig 791 in a dose of 0.3 c.c. prevented fever entirely, with the possible exception of two days when the temperature was 103 and 103.1 respectively. In the second test the same quantity of blood permitted a distinct though moderate fever for five days. Similarly, 0.3 c.c. of the blood of Guinea-pig 789 in the first test afforded greater protection than 0.6 c. c. in the second test. The conclusion that such a diminution does occur has been borne out in other experiments.

Such comparative experiments, performed at different times, are subject to possible error in that the minimum pathogenic dose of two different lots of virus may not be identical. In our experience, however, this variation has been slight and certainly would not cause greater error than would result from slight differences in the susceptibility of different animals.

An experiment similar to the one just described was carried on with Guinea-pigs 835, 836 and 837, only the essential points of which will be given.

On November 25, all three were injected with 1 c.c. of virus to

which had been added different quantities of immune blood; for 835, 0.3 c.c.; 836, 0.1 c.c., and 837, 0.05 c.c.

Guinea-pig 835 had no fever following the injection. Guinea-pig 836 showed the following course of temperature on successive days: 103.4, 103.7, 103, 103, 102.9, 103.8, 103.4, 103.8, 104.4, 104.5, 103.3, 102.8, 103.2, 102.6. The fever traceable to the virus was brief and mild in character. Guinea-pig 837 suffered severe infection with the following course of the temperature: 102.9, 102.8, 102.9, 103.1, 102.8, 105, 105.6, 105.8, 104.9, 104.6, 105.1, 104.2, 103.4, 103, 103.3, 102.6, 102.6.

They received no further inoculations of virus, hence the experiment represents an attempt to immunize by one injection.

The first comparative test of the protective powers of the bloods of the three animals was made on January 7, about two months after the injections were made. This was ample time for all the passive immunity to have disappeared, as shown by an experiment quoted earlier in this paper. Suitable quantities of the different bloods were not used to afford intelligent comparison of their properties. The second test, performed February 5, showed that 2 c.c. of the blood of 835 had no protective effect, whereas 0.8 c.c. from 836, and 0.5 c.c. from 837 showed moderate protection. In the meantime an immunity test was given to 835, which resulted in its infection with spotted fever, hence it may be concluded that the initial injection, the immunizing (?) injection, which was followed by no fever, had produced little or no immunity in this animal.

A third test of the blood of 836 and 837 showed that 0.9 c.c. from 836 did not afford complete protection, whereas 0.5 c.c. from 837 entirely prevented the development of fever with the dosage of the virus used, namely, 1 c.c. of third-day virus. This test was made three months after the immunizing injection was given.

Both of the experiments described indicate that there is a parallel between the severity of the reaction following the immunizing injection and the subsequent protective power of the blood, and it is reasonable to suppose that the degree of immunity conferred by the vaccination corresponds to a certain extent with the concentration of the protective substances which appear in the blood. This does not mean, however, that a severe or even moderate febrile reaction is necessary in

order that distinct immunity be conferred. It has happened frequently that a barely perceptible reaction is followed by the development of pronounced resistance. Two instances may be cited in which the immune blood was given a few days in advance of the virus.

On December 1, 1907, Guinea-pigs 844 and 845 received each 1 c.c. of immune blood subcutaneously. Three or four days later, respectively, each was given 1 c.c. of third-day virus (Eddy strain) subcutaneously. The temperature of Guinea-pig 844 was as follows: 102, 102.8, 103.4, 103.3, 103.2, 101.8, 103.3, 103.2, 102, 102.7. That of Guinea-pig 845 was: 102.9, 103.4, 102.7, 103.3, 101.7, 102.7, 102.9, 102.5, 102.9. Sixty-seven days later each received intraperitoneally 1 c.c. of third-day virus, one being given the Eddy and the other the Bradley strain. Neither showed the slightest febrile disturbance. Controls developed the disease typically.

HEREDITARY IMMUNITY IN VACCINATED ANIMALS.

Inasmuch as strong immunity may be produced in guinea-pigs by the method of "sero-vaccination," in which only a minimum febrile reaction is necessary, it would be expected that the immunity conferred on females in this way would be conveyed to their offspring. This would be anticipated from the fact that such a transfer occurs in the case of females which have suffered from a severe attack of the disease, as described on a previous page.

This phenomenon was noted in relation to Guinea-pig 791, whose history has been given already, and to Guinea-pig 794, which had been immunized by a series of graded injections in which the quantity of immune serum was reduced until pure virus was administered.

Two young were born to Guinea-pig 791 on December 30, 1907. This was approximately one month after she received the second injection, consisting of virus alone and about two months after the mixed injection was given. One of the young (No. 1080) when 25 days old received 0.5 c.c. of virus intraperitoneally. Its temperature and appearance were unchanged by the injection, whereas a control of the same age (No. 1087), which received the same dose died of spotted fever 10 days later. The remaining animal (1081), when 52 days old was given an intraperitoneal injection of 1 c.c. of virus. No fever resulted and the guinea-pig is still living; the control acquired a

severe infection. The same result was obtained with one of the young of Guinea-pig 794, 24 days after its birth.

The experiments quoted and others which it seems unnecessary to describe show that successful sero-vaccination of the guinea-pig is possible. We are studying this problem further to determine the applicability of the method to man, paying particular attention to methods of standardization of the virus and immune serum, the durability of the protective substances under different conditions of preservation and the constancy with which a desired reaction can be obtained. It is our intention at the same time to investigate the behavior of the monkey to this method of vaccination.

It does not follow that one can pass directly from the guinea-pig to man or from the monkey to man in sero-vaccination, using corresponding proportions of virus and immune serum. The unknown susceptibility of man in comparison with that of the monkey and guinea-pig is a serious stumbling-block in this connection. A mixture which is neutral for the guinea-pig or which produces only a slight reaction in it, may produce a severe reaction in man. The converse may also be true: that a dosage or a proportion of constituents which would excite an immunizing reaction in the guinea-pig or monkey would be without effect in man. There is also a further possibility to face, namely, that the virus, as a result of cultivation in the guinea-pig, may have undergone modifications in its virulence, whereby it may have become less virulent or more virulent for man.

Only one method could possibly be advocated at the outset; namely, to use such proportions of virus and immune serum as would leave no question as to the safety of the procedure, assuming for the time that the virus has the greatest possible virulence for man. An index of the effect of such injections could be obtained by studying the properties of the resulting serum. In the event that no antibodies were formed it would then appear justifiable to decrease the proportion of immune serum to that of virus, again studying the effect of the injection on the properties of the serum. This process could be continued until a mixture is obtained which causes the appearance of antibodies without exciting a severe reaction.

SUMMARY.

An attack of spotted fever in the guinea-pig and monkey produces a strong active immunity of long duration. This immunity is characterized by the presence of protective antibodies in the serum which may be demonstrated by injecting mixtures of virus and immune serum. The concentration of the antibodies in the blood of the immune animal undergoes a decrease in the course of several weeks.

The female that has recovered from spotted fever transmits immunity to her young. The young are immune even when the female acquired her immunity several months before impregnation. The immunity of the young does not depend on the ingestion of milk from the immune mother. The character of the inherited immunity has not yet been determined, although it is presumptively a passive immunity that differs from the passive immunity conferred by the injection of immune serum by its longer duration. The long duration of the inherited immunity may depend on the longer time required for the elimination of large quantities of protective substances.

Passive immunity may be established in the healthy guinea-pig by the injection of blood or serum from the immune guinea-pig. The immune defibrinated blood contains antibodies in such concentration that 0.1 c.c. often protects against 1 c.c. of third-day virus, representing anywhere from 30 to 100 minimum pathogenic doses. In other instances 0.3 or 0.4 c.c. of immune blood are required for this degree of protection. When 1 c.c. of strong immune blood is injected subcutaneously into healthy guinea-pigs, the passive immunity is still present in marked degree after 20 days; after 38 days it is present only in such degree that a mild course of spotted fever results when virus is injected; after 45 days it is no longer manifest. It is possible that passive immunity would not last so long if the immune blood is injected into a foreign species.

The guinea-pig may be protected against spotted fever following its inoculation with infected blood, provided the immune blood is administered on the second or third day after inoculation.

The curative power of the immune blood or serum is low and in order to produce a distinct effect it is necessary to begin its administration early in the disease and to give relatively large quantities. It exerts

a modifying effect on the severity of the infection without bringing about rapid subsidence of the symptoms.

By the method of mixed immunization or sero-vaccination, in which virus and immune blood or serum are mixed in suitable proportions, it is possible to immunize the guinea-pig by one or several injections, with the result that he is thereafter immune to infection. The blood of animals immunized in this way contains protective antibodies in fairly high concentration and in the case of females the immunity is transferred to the offspring. The quantity of antibodies produced by this method of immunization probably is in proportion to the severity of the febrile reaction which follows the administration of the immunizing dose. However, strong immunity has resulted in some instances in which the immunizing injection caused only a barely perceptible febrile reaction.

It may be possible to use the immune serum from the horse, which we have shown to be susceptible to inoculation, for the prevention of the disease in man. For this purpose the serum should be injected in sufficient quantity within two or three days following the bite of the tick. Such an injection should not be considered protective for a longer period than three weeks.

The method of sero-vaccination is not yet sufficiently perfected to warrant its application to man, but the subject is being studied further in order to determine its safety and efficiency.

There is no hope of obtaining a stronger serum for curative purposes than that yielded by an animal which has recently recovered from the disease, until the microorganism can be cultivated artificially, thus making available a desirable quantity of antigens for immunization. Even with this condition realized the results of further immunization cannot be anticipated but must await experimental determination.